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19 ABSTRACT (Continue on reverse if necessary and identify by block number) The objective of this research is to elucidate the amino acid sequences, via gene sequencing, of the adhesive proteins from several species of mussel, with the aim of understanding how these organisms attached themselves to wet surfaces. During the past year we have completed sequencing cDNA clones that code for the entire amino sequence of the adhesive protein of <i>Mytilus edulis</i> and for the C-terminal approximately 75% of the adhesive protein of <i>Geukensia demissa</i> . In addition, we have obtained sequence information on a new <i>Geukensia</i> protein that has a 47-amino acid repeat sequence. The patterns of repeat sequences in both adhesive proteins suggests to us that the conformations of the repeat peptide domains is important for function, but that the overall arrangement of the domains is less so.			
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ANNUAL PROGRESS REPORT

GRANT#: N00014-89-J-1756

R&T CODE: 441p014

PRINCIPAL INVESTIGATOR: Richard A. Laursen

INSTITUTE: Boston University

GRANT TITLE: Characterization of Marine Bioadhesive Proteins

PERIOD OF PERFORMANCE: 1 July 1989 - 30 June 1990

OBJECTIVE: To complete sequence studies on cDNA clones encoding the amino acid sequences of the adhesive proteins of the mussels, *Mytilus edulis* and *Geukensia demissa*.

ACCOMPLISHMENTS (last 12 months): During the past year we have completed determination of the amino acid sequence of the adhesive protein of the mussel, *Mytilus edulis*. The sequence consists of 911 amino acids including a 24-residue signal sequence. The amino acid sequence was obtained by sequencing a 2940-bp cDNA clone, which gave the entire coding sequence, except for the start codon. The remainder (the 5' end) was obtained from a second clone which was amplified using the polymer chain reaction. A key to obtaining large clones was transforming the cDNA library into the *rec⁻* host, *E. coli* CES201. Comparison of our sequence with a genomic sequence at obtained Genex Corp. reveals differences. Both proteins contain about 84 decapeptide and hexapeptide repeats and have identical sequences at the N-terminal and C-terminal ends, but there is a variation in the pattern of repeat units and in the sequences of some of the decapeptide repeats in the middle part of the protein.

We have also completed sequencing two cDNA clones from *Geukensia demissa*, which contain 1900 and 1550 basepairs, the larger of which codes for 557 amino acids. As reported earlier, the *Geukensia* sequence is more complex, consisting of repeats of an octapeptide, and one of two pentapeptides or a tripeptide. As with *Mytilus*, the sequences are identical at the 3' end (including the non-coding region), but the pattern of repeats differs toward the middle of the protein. We have not yet obtained the sequence of the 5' end.

Geukensia clones were selected by immunoscreening in an expression vector. One of the clones isolated by this procedure had a sequence completely different from any adhesive protein of known sequence. It had a repeat sequence of 47 residues with cysteine residues near each end of the repeat unit, suggesting multi-loop structure. Northern blot studies in which mRNA was hybridized with synthetic probes from the known adhesive protein sequence and from the new protein sequence indicated that the 47-mer repeat protein is not a part of the adhesive protein, i.e., there was no cross-hybridization. Most likely this new protein copurified with the adhesive protein used in raising antibodies. The repeat sequence suggests it is a structural protein, but a search of the protein sequence database revealed no obvious similarities with other proteins.

SIGNIFICANCE: The finding of multiple sequences for both the *Mytilus* and *Geukensia* adhesive proteins could have at least three causes: recombination during cloning, multiple genes within an organism, and population diversity. We now favor the latter explanation, though further study is required to demonstrate it. If this is the case, it suggests that the precise arrangement of peptide repeats in the protein is not very important for function. On the other hand, certain features in the repeat sequences are

essentially invariant, suggesting that the repeat domains possess a specific conformation that is required for function.

WORK PLAN (next 12 months): Inasmuch as funds for this project are now nearly exhausted, we will concentrate on writing up the results of these studies.

INVENTIONS (last 12 months): None

PUBLICATIONS AND REPORTS:

Lectures:

"Characterization of Mussel Adhesive Proteins"

ONR Contractors Meeting, Belmont, MD, 11/11/89

Materials Research Meeting, Boston, MA, 11/30/89

Hunan Biological Research Institute, Changsha, Hunan, P.R. China, 5/3/90

Department of Biology, Peking University, Beijing, P.R. China, 5/10/90

Cancer Research Institute, Bombay, India, 5/15/90

Articles:

Characterization and Structure of Mussel Adhesive Proteins, R.A. Laursen, J.J. Ou, X.T. Shen and M.J. Connors, in *Materials Synthesis Utilizing Biological Processes*, Materials Research Society, Pittsburgh (in press).

ANNUAL REPORT QUESTIONNAIRE

Principal Investigator: Richard A. Laursen

Institute: Department of Chemistry
Boston University
590 Commonwealth Ave.
Boston, MA 02215

Grant title: Characterization of Mussel Adhesive Proteins

Period of performance: 1 July 1989 - 30 June 1990

Number of publications last year: One (in press)

Number of patents/inventions: None

Total number of students/trainees: Two

How many are female? Two

How many are minority students? Two (Chinese)

How many are not US citizens? Two (PRC and ROC)

Awards/Honors to PI Elected Fellow of the American Association for
the Advancement of Science.

Equipment purchased: None

Email address: Laursen@bu-chem.bu.edu (Internet)

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HIGHLIGHTS

CHARACTERIZATION OF MARINE BIOADHESIVE PROTEINS

Richard A. Laursen
Boston University, 1990

Objectives

- Determine the amino acid sequence of the mussel adhesive proteins
- Understand how protein amino acid sequence affects function
- Use knowledge of adhesive protein structure to design wet-surface adhesives

Accomplishments

- Completed sequence (via cDNA sequencing) of *Mytilus edulis* adhesive protein
- Obtained C-terminal 75% of *Geukensia demissa* adhesive protein sequence
- Obtained partial sequence of new repetitive structural(?) protein from *G. demissa*

Adhesive protein partial sequences showing typical patterns

M. edulis

AKP S YP S TYK
AKS S YP P TYK
AKP TYK
AKP T YP S TYK
AKP S YP P TYK

G. demissa

GKP S S YDP GYK
GQQKQTGYDTGYK
GQQKQTA YDP GYK
GGVKKTGYS ADYK
GKP S S NVP GYK

Significance

- Structure of repeat peptide domain is important for function
- Pattern of repeat domains in intact protein is less important